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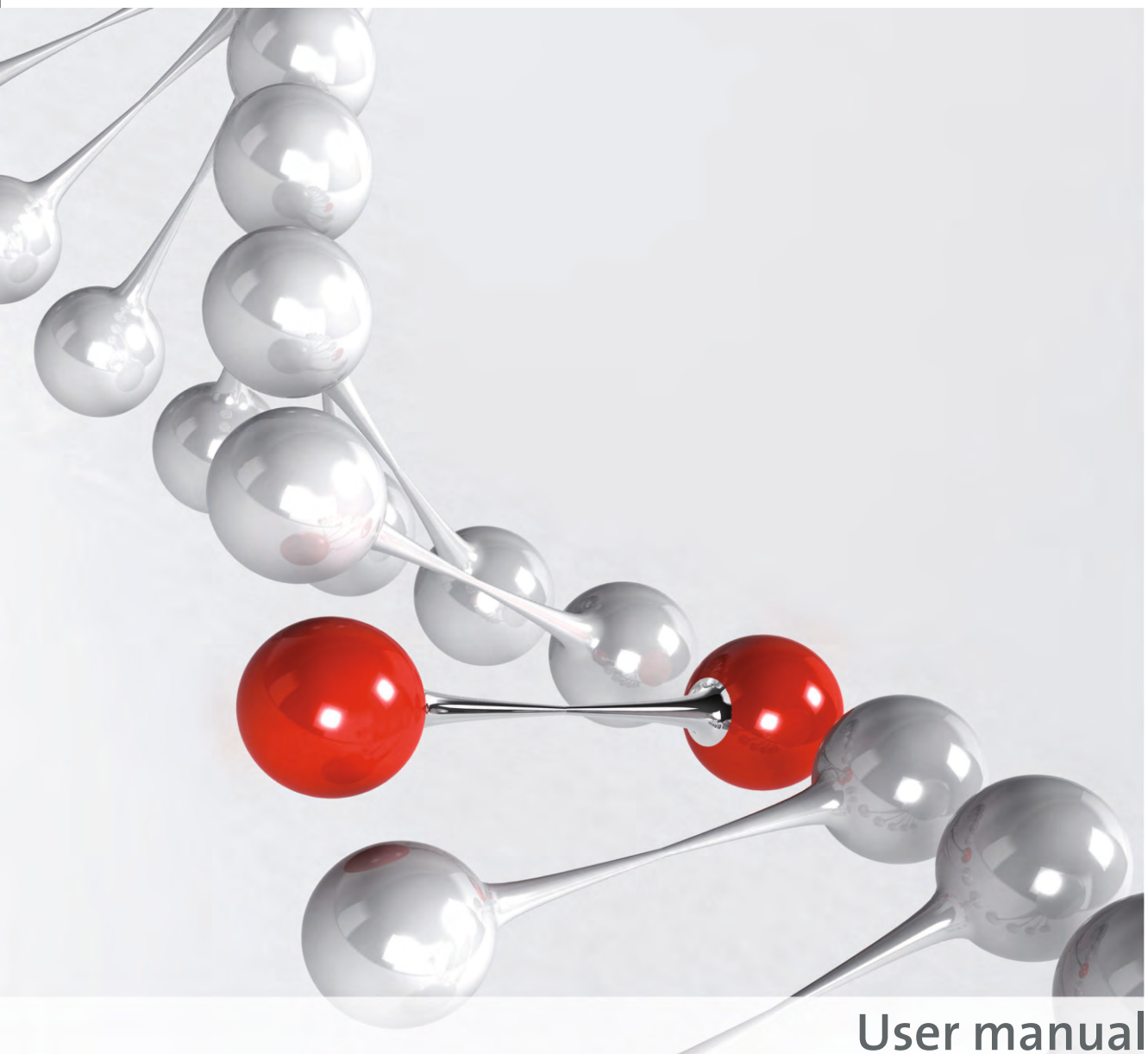

User manual

InviMag® Universal RNA Mini Kit/ KF96

for use on KingFisher® 96 and KingFisher® Flex, Thermo Fisher Scientific

for automated purification of total RNA - with a magnetic bead based DNA removal - from up to 5 x 10^6 human and animal cells, up to 1.5 ml whole blood or up to 40 mg tissue sample with magnetic beads

REF 7460300X00 STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® Universal RNA Mini Kit/ KF96

The **InviMag® Universal RNA Mini Kit/ KF96** combines the advantages of the innovative Invisorb® technology with easy handling of magnetic particles for a very efficient and reliable isolation of nucleic acids with a high purity.

The RNA-binding magnetic particles are characterized by a high surface area, uniform size distribution, good suspension stability and therefore are highly suitable for high-throughput processing.

The **InviMag® Universal RNA Mini Kit/ KF96** is applicable for isolation and purification of RNA from up to 5 x 10^6 cell pellets (fresh or frozen), from 10 - 40 mg tissue or from leucocyte pellets derived from up to 1.5 ml blood whereas the DNA is efficiently removed by the process. The kit is designed for an optimal use on the KingFisher® 96 or KingFisher Flex 96 workstation from Thermo Scientific. The interplay of the RNA extraction and purification chemistry provided by the **InviMag® Universal RNA Mini Kit/ KF96** was intensely tested and validated. Due to the high purity, the isolated RNA is ready to use for a broad panel of downstream applications or can be stored alternatively at -80°C for subsequent use.

The kit is neither suitable for isolation of RNA from blood stains, stool samples, bacteria, fungi, plants or viruses nor for purification of DNA.

Trademarks: InviMag®, Invisorb®. Registered mark, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag® and Invisorb® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of InviMag® Universal RNA Mini Kit/ KF96

Store the SNAP Solution at 4°C.
Store all other kit components at room temperature.

	1 x 96 extractions	5 x 96 extractions
Catalogue Number	7460300100	7460300200
Buffer EL conc.	2 x 30 ml	8 x 30 ml
Lysis Solution TR	60 ml	260 ml
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer R1	80 ml final volume 160 ml	2 x 125 ml final volume 2 x 250 ml
Wash Buffer R2	40 ml final volume 200 ml	4 x 50 ml final volume 4 x 250 ml
Elution Buffer R	15 ml	60 ml
2.0 ml Deep Well Plate	4	20
KF96 TipComb for DW magnets	1	5
200 µl Elution Plate	2	10
1.5 ml Receiver Tubes	2 x 50	10 x 50
Manual	1	1
Initial steps	Add 30 ml **Buffer EL** to 970 ml pure water (2x) Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer R1**. Add 160 ml of 96-100% ethanol to the bottle **Wash Buffer R2**. Mix thoroughly and always keep the bottles firmly closed!	Add 30 ml **Buffer EL** to 970 ml pure water (8x) Add 125 ml of 96-100% ethanol to each bottle **Wash Buffer R1**. Add 200 ml of 96-100% ethanol to each bottle **Wash Buffer R2**. Mix thoroughly and always keep the bottles firmly closed!

Kit contents of InviMag® Universal RNA Mini Kit/ KF96 w/o plastic

Store the SNAP Solution at 4°C.
Store all other kit components at room temperature.

	1 x 96 extractions	5 x 96 extractions
Catalogue Number	7460300150	7460300250
Buffer EL conc.	2 x 30 ml	8 x 30 ml
Lysis Solution TR	60 ml	260 ml
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer R1	80 ml final volume 160 ml	2 x 125 ml final volume 2 x 250 ml
Wash Buffer R2	40 ml final volume 200 ml	4 x 50 ml final volume 4 x 250 ml
Elution Buffer R	15 ml	60 ml
1.5 ml Receiver Tubes	2 x 50	10 x 50
Manual	1	1
Initial steps	Add 30 ml **Buffer EL** to 970 ml pure water (2x) Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer R1**. Add 160 ml of 96-100% ethanol to the bottle **Wash Buffer R2**. Mix thoroughly and always keep the bottles firmly closed!	Add 30 ml **Buffer EL** to 970 ml pure water (8x) Add 125 ml of 96-100% ethanol to each bottle **Wash Buffer R1**. Add 200 ml of 96-100% ethanol to each bottle **Wash Buffer R2**. Mix thoroughly and always keep the bottles firmly closed!
Plastic to be supplied by user (see order information)		
2.0 ml Deep Well	4	20
KF96 Tip Comb for	1	5
KingFisher 96 KF plate	2	10

Symbols

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of the **InviMag® Universal RNA Mini Kit/ KF96**, except **SNAP Solution,** should be stored at room temperature and are stable for at least 12 months under these conditions.

Room temperature (RT) is defined as range from 15 - 30°C.

SNAP Solution: The magnetic beads should be stored at 4-8°C.

Wash Buffer: Wash Buffer charged with ethanol should be stored at room temperature and should be appropriate sealed. If there are any precipitates within the provided solutions solve these precipitates by careful warming up to room temperature (up to 30°C).

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Universal RNA Mini Kit/ KF96** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Universal RNA Mini Kit/ KF96** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® Universal RNA Mini Kit/ KF96** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany +49-(0)30-9489-2901/ 2910
from abroad +49-(0)30-9489-2907/ 2903
or contact your local distributor.

Intended use

The **InviMag® Universal RNA Mini Kit/ KF96** is designed for semi-automated extraction and purification of RNA – DNA is excluded - from up to 96 samples using magnetic beads and the KingFisher 96 or KingFisher Flex 96 instrument. The supplied nucleic acid isolation protocols are capable for routinely walk-away automated preparation of RNA from fresh or frozen cultured cells, tissue samples and leukocyte pellets derived from blood. For reproducible and high yields an appropriate sample storage is essential (see “Sampling and storage of the starting material”, page 8). All utilities (reagents and plastic ware) necessary for preparation of RNA are provided by the **InviMag® Universal RNA Mini Kit/ KF96** in different package sizes.

The procedure of the **InviMag® Universal RNA Mini Kit/ KF96** is optimized for the isolation of RNA from up to 40 mg of fresh or frozen tissue sample, from up to 5 x 10^6 cells and from leukocyte pellets derived from up to 1.5 ml blood.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is neither validated for the isolation of RNA from blood stains, stool samples, bacteria, fungi, plants or viruses nor for purification of DNA.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Universal RNA Mini Kit/ KF96** procedures for residual risk materials. Therefore, liquid waste should be handled and treated accordingly to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Universal RNA Mini Kit/ KF96** to which they apply, are listed below as follows:

Lysis Solution TR and **Wash Buffer R1** contain a chaotropic salt which is an irritant. **Wash Buffer R1** and **Elution Buffer R** contain DEPC treated dd-H_2O. DEPC is inactivated by autoclaving for 20 minutes at 121°C.

Lysis Solution TR

warning

contains guanidine thiocyanate;

H302-312-332-412, EUH032, P273

Wash Buffer R1

warning

contains guanidine thiocyanate;

H302-312-332-412, EUH032, P273

H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
P273:	Avoid release to the environment.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA:	**1 – 352 – 323 – 3500**
inside of USA :	**1 – 800 – 535 – 5053**

Product characteristic of the InviMag® Universal RNA Mini Kit/ KF96

The **InviMag® Universal RNA Mini Kit/ KF96** procedure is the ideal tool for efficient extraction and purification of cell RNA using magnetic beads and the KingFisher 96 or KingFisher Flex 96. The DNA is removed during the process.

Starting Material	Yield	Time	Ratio
from 10 - 40 mg fresh or frozen tissue	up to 80 µg depending on the used tissue	about 60 min	A_{260}/A_{280} 1.7-2.1
from up to 5 x 10^6 fresh or frozen cells	up to 20 µg depending on the used cells		
from up to 1.5 ml whole blood	up to 6 µg depending on the used blood		

The RNA isolation process is based on the interaction of nucleic acids with magnetic particles under adapted buffer conditions. The KingFisher instrument performs all steps of the RNA purification procedure automatically with only minor user intervention, thus allowing safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-overs are effectively eliminated by this automated purification process.

The KingFisher® instrument uses magnetic rods to transfer the RNA-binding magnetic particles through the various purification phases: DNA-removal, RNA-binding, washing and elution. The volume of buffers and other liquids necessary for RNA isolation is reduced to a minimum. Eliminating the direct liquid handling and increasing the automation level result in a fast, reliable and robust technique. The overall efficiency speeds up the procedure.

After cell lysis outside the workstation in the optimized **Lysis Solution TR** and removal of the DNA, optimal binding conditions are adjusted by addition of **Ethanol.** The RNA, bound to the simultaneously added magnetic particles, is separated from the solution by magnetic rods controlled by the KingFisher instrument. Subsequent to the washing steps of the particle bound nucleic acids, the RNA is eluted in **Elution Buffer R**.

Due to the high purity, the eluted RNA is ready to use in a broad panel of downstream applications:

- RNA dot blots
- cDNA transcription
- Real-time PCR* (quantitative RT-PCR, like TaqMan® und LightCycler® technologies)
- Array technologies

The results from downstream applications should be interpreted with regard to other clinical or laboratory findings. To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

For the isolation of RNA from a single sample STRATEC Molecular offers the **Invisorb® Spin Cell RNA Mini Kit** or **Invisorb® Spin Tissue RNA Mini Kit** for use on a centrifuge (see Ordering information, page 24). For further information please contact: phone +49 (0) 30 9489 2901, -2910 in Germany and from foreign countries phone +49 (0) 30 9489 2907, -2903 or your local distributor.

* The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Sampling and storage of starting material

Tissues, cells and blood may be used fresh or frozen (stored at –80°C).

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Principle and procedure

The **InviMag® Universal RNA Mini Kit/ KF96** procedure comprises the following steps:

- Lysis or homogenization of the material in **Lysis Solution TR**
- Binding of the RNA to magnetic beads and removal of DNA by the contained carrier
- Washing steps and elimination of ethanol
- Elution of RNA

After lysis and removal of DNA, the RNA binds to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following three washing steps. Highly purified RNA is eluted in **Elution Buffer R**.

This manual contains 3 protocols.

Yield and quality of RNA

The amount of purified RNA in the **InviMag® Universal RNA Mini Kit/ KF96** procedure depends on the type and condition of the cells or tissues used.

Yield and quality of isolated RNA is suitable for any downstream processing.

Important notes

Important points before starting a protocol

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard gloves if they get contaminated.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

Before starting a run, bring all reagents to room temperature. When necessary, gently mix and redissolve any precipitates by warming up to 30°C. Swirl gently to avoid foaming.

Lysis Solution TR, and **Elution Buffer R** are ready to use.

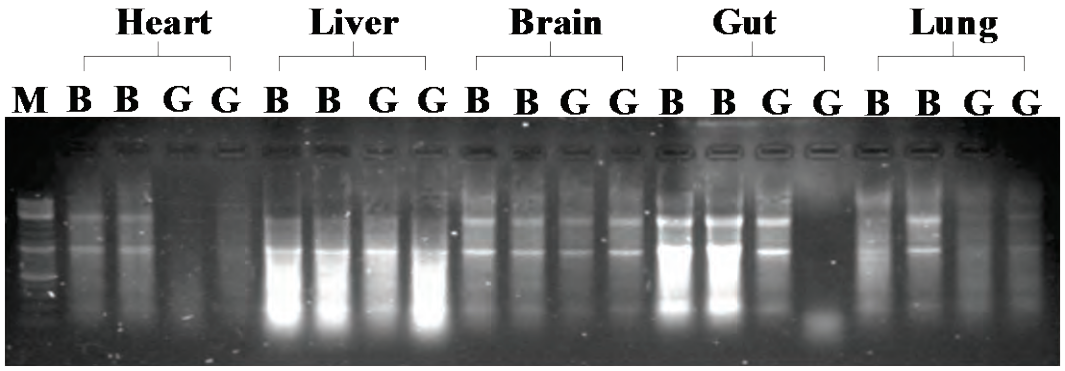
1x 96 extractions
Add 30 ml **Buffer EL** to 970 ml pure water (2x)
Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer R1**.
Add 160 ml of 96-100% ethanol to the bottle **Wash Buffer R2**.
Mix thoroughly and always keep the bottles firmly closed!
5x 96 extractions
Add 30 ml **Buffer EL** to 970 ml pure water (8x)
Add 125 ml of 96-100% ethanol to each bottle **Wash Buffer R1**.
Add 200 ml of 96-100% ethanol to each bottle **Wash Buffer R2**.
Mix thoroughly and always keep the bottles firmly closed!

Reagents and equipment to be supplied by user

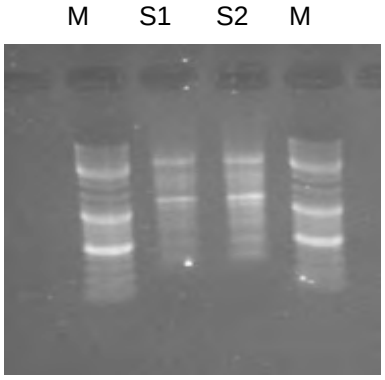
- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- ddH_2O
- Vortexer
- 96 - 100% Ethanol

Product validation

Figure 1: The quality and yield of the **InviMag® Universal RNA Mini Kit/ KF96** was ensured by multiple testing. Figure 1 shows the result of a typical RNA extraction of 10-20 mg different tissues using two types of homogenizers.

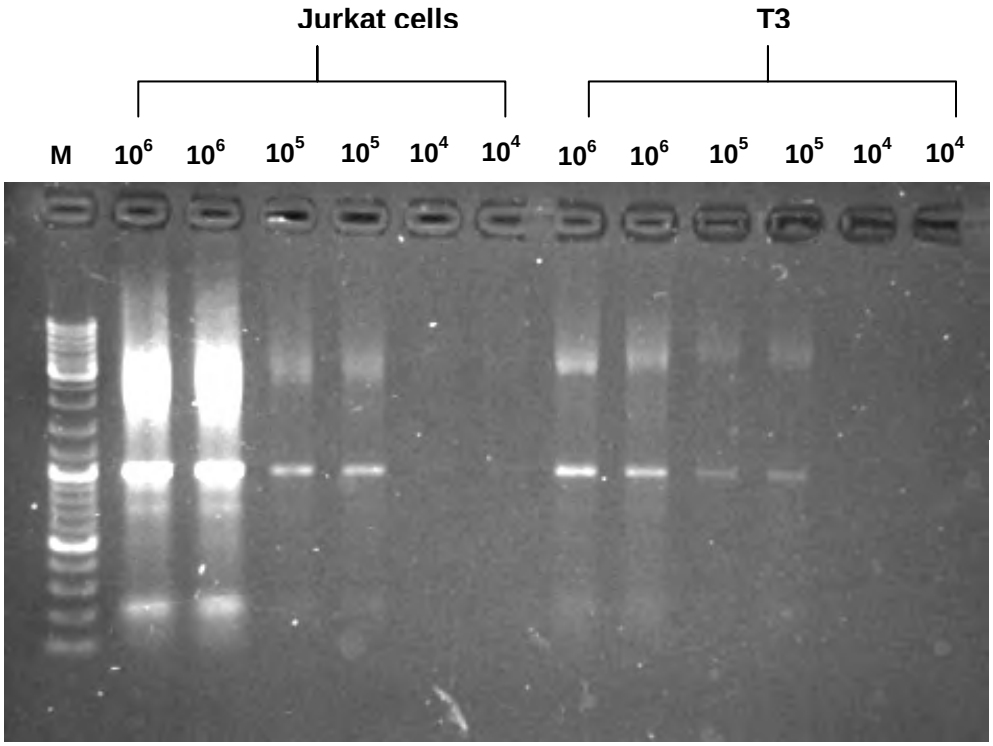


M = Marker „Generuler™“ (Fermentas), B = Bulltet Blender (NEXT), G = Gyrator (STRATEC Molecular); 10 µl sample, respectively, was used for gel analysis



M = Marker „Generuler™“ (Fermentas, 3.5 µl), S = Sample

Figure 2: shows the typical result of a RNA preparation of leukocytes derived from 1.5 ml whole blood using with the **InviMag® Universal RNA Mini Kit/ KF96.**



M = Marker „Generuler™“ (Fermentas);
10 µl sample, respectively, was for gel analysis

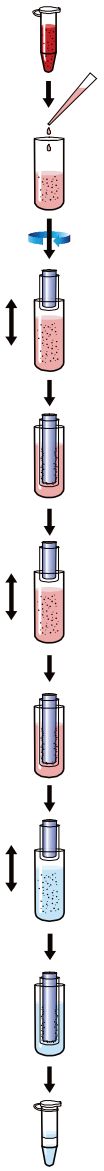
Figure 3: shows a common result of a RNA preparation of two cell lines using different amount of cells performed with the **InviMag® Universal RNA Mini Kit/ KF96.**

Scheme of the InviMag® Universal RNA Mini Kit/ KF96

Please read protocols carefully prior to the start of the preparation.

Prefill all KingFisher plates with the needed buffers and appropriate volumes as shown below.

Tip Plate:	Place a KF96 Tip Comb for DW magnets on a 200 µl Elution Plate*
Binding Plate:	Add 400 µl **Ethanol** and 20 µl **SNAP Solution**
Washing Plate 1:	Add 800 µl **Wash Buffer R1** to 2.0 ml Deep Well Plate
Washing Plate 2:	Add 800 µl **Wash Buffer R2** to 2.0 ml Deep Well Plate
Washing Plate 3:	Add 800 µl **Wash Buffer R2** to 2.0 ml Deep Well Plate
Elution Plate:	Add 100 µl **Elution Buffer R** to a KF96 Plate (same size as Tip Plate)



Sample Lysis

- Transfer the sample into a 1.5 ml reaction tube and add 500 µl LysisBuffer TR and 5 µl DTT (1 M stock solution, not provided). Disrupt the sample by use of a mortar and pestle or a comparable electronic device.
- After lysis, centrifuge each sample for 1 min at 13000 rpm (table centrifuge) and transfer the supernatant to the Binding plate containing 400 µl ethanol and 20 µl SNAP solution.
- Start the run bei either choosing the program “InviMag RNA Universal KF96” or “InviMag RNA Universal KFflex 96” and follow the instructions shown on the KingFisher instrument display.

RNA Binding to magnetic beads while DNA is excluded

Magnetic separation

Washing of the particle fixed RNA

Magnetic separation

Elution of RNA

Magnetic Separation

Pure RNA

*** Elution Plates KF96 and Tip Plates are identically. Use one provided Elution Plate as a Tip Plate.**

Protocol 1: Extraction of total RNA from fresh cultured cells or frozen cell pellets

Please read the instructions carefully and conduct the prepared procedure.

Total RNA extraction from human and animal cell culture

1. Harvesting cells

Cells grown in suspension:	Spin down up to 1x10^6 cells for 5 min at 1.500 rpm. Discard the supernatant and remove all media completely.
Cells grown in a monolayer:	In *large culture vessels* (dishes > ∅ 35 mm, flasks > 12.5 cm^2) detach cells by trypsination. Transfer the cells into a centrifugation tube and sediment by centrifugation at 1500 rpm for 5 min. Remove the supernatant completely. In *small culture vessels* (96-, 24-, 12-, 6-well plates, ∅ 35 mm dishes, 12.5 cm^2 flasks) discard the media completely and continue with the lysis immediately.

Important: *Incomplete removal of the cell culture media will inhibit the lysis and dilution of the lysate will affect the binding of RNA to the magnetic beads.*

2. Cell Disruption

a) Cell pellet:

To loosen the cell pellet flick the tube and add **Lysis Solution TR** with **DTT** (see tab. 1; DTT is not supplied within the kit). Mix thoroughly by pipetting up and down until no cell clumps are visible. Incubate for 2 min at room temperature before proceeding with the next step.

Centrifuge the suspension for 1 min at maximum speed (e.g. 13.000 rpm on a table centrifuge). Carefully transfer the supernatant to the prepared Binding Plate without disturbing the pellet.

Table 1:

Lysis Solution TR	1 M DTT	Number of pelleted cells
500 µl	5 µl	up to 1x10^6 cells

b) Monolayer cells:

Add **Lysis Solution TR** with **DTT** (see tab. 2; DTT is not supplied within the kit) to the monolayer cells. Collect the cell lysate with a rubber policeman. Mix thoroughly by pipetting up and down until no cell clumps are visible. Incubate for 2 min before proceeding with the next step.
Centrifuge the suspension for 1 min at maximum speed (e.g. 13.000 rpm on a table centrifuge). Carefully transfer the supernatant to the prepared Binding Plate without disturbing the pellet.

Table 2:

Lysis Solution TR	1 M DTT	Size of the culture vessels
500 µl	5 µl	96-; 24-; 12-well-plates

Protocol 2: Extraction of RNA from fresh or frozen tissues

Please read the instructions carefully and conduct the prepared procedure.

The **disruption procedure**, the breakage of intercellular matrix, like cells walls, organelles and plasma membranes, is necessary to release the nucleic acids contained in the cell, thus inefficient disruption decreases the RNA yield. Different samples require different methods to achieve complete disruption.

The **homogenization** means the reduction of the viscosity of the lysate after disruption. Contaminating genomic DNA and other cellular components of high molecular weight are sheared to form a homogenous lysate. Incomplete homogenization results in an inefficient binding of RNA to the magnetic beads and therefore significantly reduces yields.

It is possible to use a commercially available bead mill in combination with or without beads for the disruption and homogenization of the starting material. Alternatively, the starting material can be grinded to a fine powder in liquid nitrogen using a mortar and pestle.

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step.

Rotor–stator homogenizers and bead mills are used for simultaneously disruption of samples. In contrast, the use of a mortar and pestle only drisrupt the sample.To achieve a complete homogenization, a separate step is needed. This can be accomplished by use of a shredder spin column or a 20 gauge needle.

a) Disruption and homogenization using rotor-stator homogenizer

Using a rotor-stator homogenizer in combination with **Lysis Solution TR** and **DTT** (see Table 1, page 13) disrupts and simultaneously homogenizes in 5-90 s, depending on the toughness of the sample.

b) Disruption and homogenization using a bead mill (e.g. Gyrator*)

With bead-mills, tissue samples can be disrupted by rapid agitation in the presence of **Lysis Solution TR and DTT** (see Table 1, page 13). Disruption and simultaneous homogenization occurs by shearing and crushing with the beads as they collide with the cells. The disruption efficiency is influenced by:

- Size and Composition of beads
- Ratio of buffer to beads
- Amount of starting material
- Speed of the mill
- Disintegration time

A mixture of Zirconia beads with a diameter of 7 x 0.7 mm (Zirconia Beads I) and 2 x 2.4 mm (Zirconia Beads II) are the optimal mixture for tissue disruption. All other disruption parameters should be determined empirically for each application.

c) Disruption and homogenization using a mortar and pestle

To disrupt tissue using a mortar and pestle, freeze the sample immediately in liquid nitrogen, and grind it to a fine powder. Transfer the suspension (tissue powder and liquid nitrogen) into an RNase free tube. Allow the liquid nitrogen to evaporate, but do not allow the sample to thaw. After evaporation of the liquid nitrogen add 500 µl **Lysis Solution TR** with **DTT** (see Table 1, page 13).

After addition of Lysis solution (end of step a, b or c) vortex or pipet up and down several times to solve the cell pellet completely. Incubate for 2 min at RT before proceeding with the next step.
Centrifuge the suspension for 1 min at maximum speed (e.g. 13.000 rpm on a table centrifuge). Carefully transfer the supernatant to the prepared Binding Plate without disturbing the pellet.

Protocol 3: RNA extraction from up to 1.5 ml whole blood

Please read the instructions carefully and conduct the prepared procedure.

Note: *The centrifugation steps were performed with the **Centrifuge 5415 D from Eppendorf**. The incicated rpm amounts are referring to this centrifuge.*

1. Lysis of Erythrocytes

Add 10 ml of cold (4°C) prepared **Buffer EL** (to 0.5 - 1.5 ml of whole blood (max. 1 x 10^7 leukocytes) in a 15 ml tube (e.g. 15 ml Falcon Tube, not provided). Mix shortly but completely by vortexing.

Incubate on ice for 15 min. Mix briefly by vortexing two times during incubation.

Note: *The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, the incubation time can be extended to 20 min.*

Centrifuge at 1.700 x *g* (3.000 rpm) for 5 min at 4°C, carefully remove and discard the supernatant completely without disturbing the visible, white leukoytes pellet.

Note: *Leukocytes will form a pellet after centrifugation. Ensure that the supernatant is completely removed by aspiration. Trace amounts of erythrocytes, which give the pellet the red tint, will be eliminated by following washing steps.*

Important: *If fresh blood samples (up to 4 h after taking the sample) are used, extend the lysis time of erythrocytes to 30 min, that the total lysis time is at least 45 min. Please keep in mind, that up to 1.5 ml whole blood can be processed. If the expected amount of leukocytes is more than 1x10^7, reduce the starting volume of the blood sample.*

Add 5 ml of cooled **Buffer EL** to the cell pellet, vortex shortly and centrifuge at 1.700 x *g* (3.000 rpm) for 5 min at 4°C. Carefully remove the supernatant completely.

Note: *Incomplete removal of the supernatant will interfere with lysis and subsequent binding of the RNA to magnetic particles, resulting in lower yield and purity.*

2. Leukocyte disruption

Important: *Before starting with step 2 vortex the **Lysis Solution TR** vigorously and avoid contaminations with RNase.*

Resuspend the cell pellet from step 1 in 500 µl **Lysis Solution TR** with **DTT** (see Table 2)**.** Vortex or pipet up and down several times to solve the cell pellet completely. Incubate for 2 min before proceeding with the next step.

Centrifuge the suspension for 1 min at maximum speed (e.g. 13.000 rpm on a table centrifuge). Carefully transfer the supernatant to the prepared Binding Plate without disturbing the pellet.

*) **Gyrator, UNIPREP. universal 3-D Vortexer for Microtubes, from UniEquip GmbH (phone +49(0)89-8575200, phone +49(0)89- 8575205, Fax: +49(0)89-8561304 email: info@uniequip.de)**

****) Please refer to suppliers' guideline for further details**

Starting a run on a KF96 or KFflex 96 instrument

I. Preliminary Steps to process the sample onto the KingFisher System

Important: *For working with the KF96 or KFflex 96 system please carefully read the documents supplied by the instrument manufacturer!*

1. Turn on the KF96 / KFflex 96 system

2. Prefill the Deep Well Plates with the appropiate buffers and volumes.

Notes*:*

- *In case of not starting a run immediately, please avoid evaporation of the prefilled buffer components by sealing the Deep Well Plates with a sealing foil or parafilm!*
- *Mix the bottle with the **SNAP Solution** by vigorously vortexing before use!*
- *Use one of the provided Elution Plate as a Tip Comb Plate. These plates are identical.*

Tip Plate:	Place a KF96 Tip Comb for DW magnets on a 200 µl Elution Plate.
Binding Plate:	Transfer the supernatant of the lysed sample into the cavity of the Binding Plate prefilled with 400 µl **Ethanol** and 20 µl **SNAP Solution**.
Washing plate_1:	Pipet 800 µl **Wash Buffer R1** into the cavities of a Deep Well Plate
Washing plate_2:	Pipet 800 µl **Wash Buffer R2** into the cavities of a Deep Well Plate
Washing plate_3:	Pipet 800 µl **Wash Buffer R2** into the cavities of a Deep Well Plate
Elution Plate:	Pipet 100 µl **Elution Buffer R** into the cavities of the Elution Plate

3. Choose the program “InviMag Uni RNA_KF96” (KF96 protocol) or “InviMag Uni RNA_KFflex96” (KFflex 96 protocol) and press the “START” button.

4. Insert the prefilled plates into the right position of the instrument surface by following the specification given on the display and confirm every step by pressing the “START” button. After all prefilled plates have been loaded, press the “START” button again to execute the run.

Important Notes: *At the end of the extraction protocol the isolated RNA is loacted in the Elution Plate. In case of long-term storage we recommend to to seal the plate or transfer the isolated RNAs into a 1.5 ml reaction tubes and freeze them at -80°C.*

II. Extraction steps automatically performed on the KingFisher system

Binding of the DNA

Automatically sample mixing for 5 min. SNAP separation. SNAP transfer to Washing Plate 1.

First Washing

Automatically sample mixing for 2 min. SNAP separation. SNAP transfer to Washing Plate 2.

Second Washing

Automatically sample mixing for 1 min. SNAP separation. SNAP transfer to Washing Plate 3.

Third Washing and Drying

Automatically sample mixing for 1 min. SNAP separation. Drying the SNAP outsight Washing Plate 3 for 7 minutes. SNAP transfer to Elution Plate.

Elution of the DNA

Automatically sample mixing for 10 minutes. SNAP separation and removal into Washing Plate_3 (disposal). The extracted DNA can now be transferred into 1.5 ml reaction tubes.

Important Notes: *After the run, the Elution Plate contains the extracted RNA. Store the RNA under adequate conditions. We recommend to transfer the extracted RNA into an 1.5 ml reaction tube for further storage and freeze the DNA at -20°C. Alternatively, the Elution Plate can be sealed and stored at –80°C.*

If the extracted RNA contains carryover of magnetic particles, transfer the RNA into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 min and transfer the RNA-containing supernatant into a new tube.

For self-programming of the KingFisher 96 instrument

Protocol: InviMag_Uni_RNA_KF96

[PROTOCOL PROPERTIES]

Name = InviMag_Uni_RNA_KF96
Protocol template version = 2.6.0
Instrument type = KingFisher 96
Description = KingFisher 96 (Thermo Electron) protocol for Isolation of total RNA from cells or tissue with the InviMag Universal RNA Mini kit
Kit = InviMAG Universal RNA Mini Kit/ KF96/ KFflex96
Plate layouts = Tip Plate, Binding Plate, Wash 1, Wash 2, Wash 3, Elution Plate

[PLATE LAYOUTS]

TIP PLATE

Plate type = KingFisher 96 plate
Plate change message = Change Tip Plate
A:
- EMPTY

BINDING PLATE

Plate type = Thermo DW
Plate change message = Change Binding
A:
- volume = 505, name = Lysed Sample
- volume = 400, name = Ethanol
- volume = 20, name = SNAP Solution

WASH 1

Plate type = Thermo DW
Plate change message = Change Wash 1
A:
- volume = 800, name = Wash Buffer R1

WASH 2

Plate type = Thermo DW
Plate change message = Change Wash 2
A:
- volume = 800, name = Wash Buffer R2

WASH 3

Plate type = Thermo DW
Plate change message = Change Wash 3
A:
- volume = 800, name = Wash Buffer R2

ELUTION PLATE

Plate type = KingFisher 96 plate
Plate change message = Change Elution
A:
- volume = 100, name = Elution Buffer R

[STEPS]

BIND

Step parameters

- Name = Binding
- Plate = Binding Plate

Beginning of step:

- Premix = Yes

Bind parameters:

- Bind time = 5min 0s, speed = Slow

End of step:

- Collect beads = Yes, count = 5

WASH 1

Step parameters

- Name = Washing Step 1
- Plate = Wash 1

Beginning of step:

- Release = Yes, time = 30s, speed = Medium

Wash parameters:

- Wash time = 2min 0s, speed = Medium

End of step:

- Collect beads = Yes, count = 3

WASH2

Step parameters

- Name = Washing Step 2
- Plate = Wash 2

Beginning of step:

- Release = Yes, time = 30s, speed = Medium

Wash parameters:

- Wash time = 1min 0s, speed = Medium

End of step:

- Collect beads = Yes, count = 3

WASH 3

Step parameters

- Name = Washing Step 3
- Plate = Wash 3

Beginning of step:

- Release = Yes, time = 30s, speed = Medium

Wash parameters:

- Wash time = 1min 0s, speed = Medium

End of step:

- Collect beads = Yes, count = 3

DRY

Step parameters

- Name = Drying
- Plate = Wash 3
- Dry time = 7min 0s

- Tip position = Outside well

ELUTION

Step parameters

- Name = Elution
- Plate = Elution Plate

Beginning of step:

- Release = Yes, time = 30s, speed = Medium

Elution parameters:

- Elution time = 10min 0s, speed = Slow
- Heating = No

Remove beads:

- Remove beads = Yes, collect count = 5, disposal plate = Wash 3

For self-programming of the KingFisher Flex 96 instrument

Protocol: InviMag_Uni_RNA_KFflex96

[PROTOCOL PROPERTIES]

Name = InviMag_Uni_RNA_KFflex 96
Protocol template version = 3.1
Instrument type = KingFisher Flex 96
Description = KingFisher Flex 96 (Thermo Electron) protocol for Isolation of total RNA from cells or tissue with the InviMag Universal RNA Mini kit
Kit = InviMAG Universal RNA Mini Kit/ KF96/ KFflex 96
Plate layouts = Tip Plate, Binding Plate, Wash 1, Wash 2, Wash 3, Elution Plate

[PLATE LAYOUTS]

TIP PLATE
Plate type: KingFisher 96 plate

Reagents: <empty>

BINDING PLATE
Plate type = KingFisher 96 DW plate

Reagents:

Name: Sample
Volume [µl]: 505
Type: Sample

Name: EtOH, abs.
Volume [µl]: 400
Type: Reagent

Name: SNAP Solution
Volume [µl]: 20
Type: Reagent

WASHING PLATE 1
Plate type: KingFisher 96 DW plate

Reagents:

Name: Wash Buffer R1
Volume [µl]: 800
Type: Reagent

WASHING PLATE 2
Plate type: KingFisher 96 DW plate

Reagents:

Name: Wash Buffer R2
Volume [µl]: 800
Type: Reagent

WASHING PLATE 3
Plate type: KingFisher 96 DW plate

Reagents:

Name: Wash Buffer R2
Volume [µl]: 800
Type: Reagent

ELUTION PLATE
Plate type = KingFisher 96 plate
Reagents:

Name: Elution Buffer R
Volume [µl]: 100
Type:Reagent

[STEPS]

TIP PLATE:
Pick-Up plate: Tip Plate
Leave plate: Tip Plate

BINDING PLATE

Beginning of step:
Precollect: No
Release beads: No

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 5
Collect time [s]: 2

WASHING PLATE 1

Beginning of step:
Precollect: No
Release time [hh:mm:ss] 00:00:30
Release speed: Medium

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:02:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

WASHING PLATE 2

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium
Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

WASHING PLATE 3

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

DRYING

Plate: Washing Plate 3
Dry time [hh:mm:ss]: 00:07:00
Tip position: Outside well/tube

ELUTION

Beginning of step:

Precollect: No
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:10:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 5
Collect time [s]: 2

REMOVE BEADS

Plate: Washing Plate 3
Release time [hh:mm:ss]: 00:00:30
Release speed: Fast

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted RNA	insufficient lysis	increase lyses time, but prevent too long lyses times because this also decrease yield reduce amount of starting material
	incomplete elution	take higher volume of **Elution Buffer R**, be sure you pipet the **Elution Buffer R** with the right amount to the right position
	low amount of **SNAP Solution**	mix **SNAP Solution** thoroughly before use
low concentration of extracted RNA	too much **Elution Buffer**	elute the RNA with lower volume of **Elution Buffer R**
	incorrect storage of starting material	ensure that the storage of starting material was correctly avoid (repeated) thawing of the material
	incorrect **Wash Buffers**	make sure that the correct amount of ethanol is added to the **Wash Buffers** and store them correctly
degraded RNA	incorrect storage of starting material	ensure that the storage of starting material is correctly avoid thawing of the material
	old material	ensure that the starting material is fresh or stored under appropriate condition (for long time storage at –80°C) avoid thawing of the material
RNA does not perform well in downstream-applications (e.g. real-time RT-PCR or RT-PCR)	ethanol carryover during elution	increase drying time for removing of ethanol
	salt carryover during elution	check up the **Wash Buffers** for salt precipitates. If there are any precipitates, solve these precipitates by careful warming ensure that the **Wash Buffers** are at room temperature
low A_{260}:A_{280} ratio from UV measurement, eluted RNA is brown colored	small part of the magnetic particles are left in the elution	centrifuge down at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher Software Version 2.6 and 3.1

The KingFisher Software 2.6.2 was used for the creation of the KingFisher 96 protocols whereas the Software 3.1 was used for creation of KingFisher Flex 96 run files. The user can either transfer the protocol onto the workstation or run the the protocol directly from the software. Be aware that directly run protocols are not stored in the workstation memory.

If you don´t have the correct KingFisher software installed on your computer, please call your local ThermoFisher distributor for an update.

Note: ***Please keep in mind that software version 2.6 and 3.1 are not compatible! It is not possible to run a procotol created in version 2.6 under version 3.1 and vice versa!***

Minimal PC Requirements for KingFisher Software 2.6 and 3.1

PC requirements	
Interface	Serial communication port via a RS-232 full duplex interface
Supported operating systems	Microsoft Windows 2000 Microsoft Windows XP Professional
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 700 Mhz recommended
Memory	220 MB RAM recommended
Serial ports available	1
Pointing device	Mouse or equivalent is necessary
CD-ROM drive	1
Monitor / color settings	SVGA monitor with at least 1024 x 768 resolution and at least a 16-bit color environment
Service packs installed	Microsoft Windows 2000: Service Pack 4 (or greater) Microsoft Windows XP Professional: Service Pack 2 (or greater)
Browser	Microsoft Internet Explorer 6.0 (or greater) installed

If you do not have the correct Service Packs installed, you can download them from the Microsoft web pages: http://www.microsoft.com/.

General notes on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases has to be reduced as much as possible Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification is to be carried out.

All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving alone will not completely inactivate many RNases. Oven baking will both inactivate RNases and ensure that no other nucleic acids (such as Plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware has to stand 12 hours at 37°C and then autoclave or heat to 100°C for 15 min to remove residual DEPC.

- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All buffers must be prepared from DEPC-treated RNase-free ddH_2O.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Change gloves frequently and keep tubes closed.
- All centrifugation steps are carried out at room temperature
- To avoid cross contamination cavity seams shouldn't be moisted with fluid.
- Reduce the preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase free).
- Keep isolated RNA on ice.
- Do not use kit components from other kits with the kit you are currently using, unless the lot numbers are identical.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.

This kit should only be used by trained personnel.

Storage of RNA

Purified RNA can be stored at -80°C and is stable for several years at this condition.

Quantification of RNA

The concentration of RNA should be determinate by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. Readings should be greater than 0.10 to ensure significance. An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml. This relation is valid only for measurements at neutral pH. The ratio between absorbance values at 260 nm and 280 nm gives an estimate of RNA purity (see below).

When measuring RNA samples, make sure that cuvettes are RNase-free, esp. if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes

with 0.1 NaOH, 1 mM EDTA followed by washing with RNase-free water. Use the buffer in which the RNA is diluted for calibration of the spectrophotometer.

An example of the calculation involved in RNA quantification:

- Volume of RNA sample: 100 µl
- Dilution = 20 µl of RNA sample + 180 µl of 10 mM Tris/HCl pH 7.0 (1/10 dilution).
- Measure absorbance of diluted sample in a 0.2 ml cuvette (RNase free): A_{260} = 0.2

Concentration of the RNA sample = 40 µg/ml * A_{260} * dilution factor

= 40 µg/ml * 0.2 * 10

= 80 µg/ml

Total amount = concentration * volume of sample in ml

= 80 µg/ml * 0.1 ml

= 8 µg of RNA

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to the contaminants that absorb in the UV, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in lower A_{260}/A_{280} ratio and reduced sensitivity to protein contaminations.* For accurate values, it is recommend to measure absorbance in 10 mM Tris Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9-2.1** in 10 mM Tris/HCl pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution.

For determination of the RNA concentration, however, it is recommend diluting the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 40 µg/ml of RNA) is based on an extinction coefficient calculated for RNA at neutral pH.

*) Wilfinger, W.W.,Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Bio Techniques 22, 474

**) Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris/HCl, pH 7.5) with some photospectrometers.

Ordering information

Product	Package size	Catalogue No.
InviMag® Universal RNA Mini Kit/ KF96	1 x 96 preparations	7460300100
InviMag® Universal RNA Mini Kit/ KF96	5 x 96 preparations	7460300200
InviMag® Universal RNA Mini Kit/ KF96 w/o plastic	1 x 96 preparations	7460300150
InviMag® Universal RNA Mini Kit/ KF96 w/o plastic	5 x 96 preparations	7460300250

KingFisher 96 and consumables

KingFisher 96, Magnetic Particle Processor,100-240V,50/60Hz	5400500
KingFisher 96 Head for Deep Well plate	24073430
KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box	97002514
KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box	97002524
KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box	97002534
KingFisher 96 KF plate (200ul) 48 plates / box	97002540
Microtiter deep well 96 plate, 50 plates/box	95040450

Related products	Package size	Catalogue No.
InviTrap® Spin Universal RNA Mini Kit	50 preps	1060100200
InviTrap® Spin Universal RNA Mini Kit	250 preps	1060100300
InviTrap® Spin Cell RNA Mini Kit	50 preparations	1061100200
InviTrap® Spin Cell RNA Mini Kit	250 preparations	1061100300
InviTrap® Spin Tissue RNA Mini Kit	50 preparations	1062100200
InviTrap® Spin Tissue RNA Mini Kit	250 preparations	1062100300

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